SPONTANEOUS HYBRIDS IN DODECATHEON (PRIMULACEAE)

Joyce F. Goodwin
Department of Life and Physical Sciences
Allan Hancock College, Santa Maria, California 93454

When geographically distinct species grow in unusually close proximity, interspecific hybridization may occur and a hybrid swarm may be produced. This appears to be occurring with two species of *Dodecatheon* on Table Mountain in Butte County, California. One of these, *D. clevelandii* Greene ssp. *patulum* (Greene) H. J. Thomps. is growing out of its usual elevational range, very near a population of *D. hendersonii* Gray ssp. *parvifolium* (Kunth) H. J. Thomps. Certain plants in the *D. clevelandii* population are of unusual morphology and coloration for this species and are hypothesized by the author to be hybrids. In his review of *Dodecatheon,* Thompson (1953) included an analysis of artificial interspecific hybridization. He demonstrated that a cross producing vigorous hybrids is possible between these two species.

A relatively high degree of morphological similarity between two species, as is exhibited by *D. hendersonii* and *D. clevelandii,* may make morphological investigation alone inadequate for delineation of taxa, including hybrids. This study has utilized chromatography in addition to morphology in analyzing putative hybrids and their parentage.

Materials and Methods

The study site is located on Table Mountain, 5.1 km northeast of Cherokee Road from its intersection with Table Mountain Road in Oroville, Butte County, California. Two distinct habitats are represented by an open, grassy pasture adjoining an oak woodland. *Dodecatheon clevelandii* (ca 300 plants) grows in the meadow, in full sun. At this site, it is out of its usual elevational range; it is normally confined to grassy meadows on the Sacramento Valley floor (ca 67 m), whereas on Table Mountain it is found at a much higher elevation (ca 260 m). At this site, *D. hendersonii* (ca 200 plants) is confined to the more shady oak woodland, which is its normal habitat. The putative hybrids are found within the *D. clevelandii* population and near the ecotone area, also in full sun. Other populations of these two species were also studied in Butte County for morphological and chemical comparison with the Table Mountain plants (see Goodwin, 1973).

Morphological analyses were made of approximately 30 plants of each species and of nine of the dozen or so putative hybrids found during the study. Putative hybrids are designated A, B, C, 1a, 1b, 2a, 2b, 3, 4, 6, and 7. Chromosome counts for the two species (four plants each) were made from propiono-carmine squashes of anthers fixed in ethanol:acetic acid

(3:1, v:v). These slides were made permanent and have been retained by the author. Fresh pollen from ten plants of each species and six putative hybrids was subjected to aniline blue-lactophenol stain to indicate percentage of presumably viable pollen; 500 pollen grains per plant were counted. Hauser and Morrison (1964) note that results of this technique tend to give results of slightly higher percentages than the nitro-blue tetrazolium technique they employed, and which they feel to be more accurate. However, the differences in the results of these two techniques were consistently small in their work, and the aniline blue-lactophenol technique was chosen because of its simplicity. Voucher specimens for this study have been deposited in the herbarium of California State University, Chico.

Thin-layer chromatography was used to separate compounds in leaf extracts. Dried leaves from pressed plants were soaked for 18–24 hours in methanol with 0.5 percent HCl. This extract was spotted on silica gel thin layers (SilicAR TLC - 7G, Mallinckrodt). Chromatographs were developed with solvents in two phases: the first phase consisted of t-butanol, acetic acid, and water (3:2:2, v:v). The second phase consisted of acetic acid and n-propanol (1:3, v:v). Each phase was run for 10 cm. Plates were dried, and then exposed to ultraviolet light to make non-pigmented spots fluoresce; they were then treated with ammonia vapor and again exposed to ultraviolet light.

Results

Morphological characteristics of both species and the putative hybrids are summarized in Table 1. Morphologically, all *D. clevelandii* populations studied were very similar. Individuals with pink-tipped corollas were very rare (only three were found in the entire study) and resembled the individuals with white corollas in all other respects. The *D. hendersonii* populations were also very similar, with the exception of corolla coloration. Some populations had a high frequency of the darker magenta color, while others had more individuals with the lavender coloration. Only one totally white individual was found during this study; this plant resembled other plants in that population in all other respects. Putative hybrid plants were distinguished by their pink and white corollas, the condition of the maroon band on the corolla tube, and the absence of the yellow anther dot (see Table 1). No individuals in any *D. hendersonii* population showed the two-toned corolla coloration and maroon band found in the putative hybrids, and no individuals in any *D. clevelandii* population studied showed the absence of the yellow anther dot. For these reasons, the putative hybrids were rather easy to distinguish once they had flowered. Morphological variation of the Table Mountain plants is summarized in Figure 1.

Chromosome counts of both species established that plants with a haploid number of $n = 22$ are present in both species at the Table Moun-

TABLE 1. MORPHOLOGICAL COMPARISONS OF TWO SPECIES OF DODECATHEON AND PUTATIVE HYBRIDS. Samples for the species came from several populations in Butte County (see Goodwin, 1973, for localities).

Character	*D. hendersonii*	*D. clevelandii*	Putative hybrids
Scape height	10–31 cm, avg. 18.4	6–14 cm, avg. 9.7	8.5–18.5 cm, avg. 11.9
Leaf morphology	Spatulate to elliptic	Spatulate to oblanceolate	Variable; tends to resemble *D. clevelandii*
No. flowers per umbel	1–7, avg. 3.9	1–4, avg. 2.2	1–3, avg. 1.9
Corolla-segment coloration	Lavender to magenta, rarely white	White to cream, rarely pink-tipped	Pink-tipped to almost totally pink
Nature of maroon band on corolla-tube	Indented, pointing toward anthers; narrow	Indented, away from anthers; wide	Indented, away from anthers; variable width
Filament-tube width with respect to anther whorl	Narrower	Equally wide	Variable
Yellow dot at base of anthers	Absent	Present	Absent

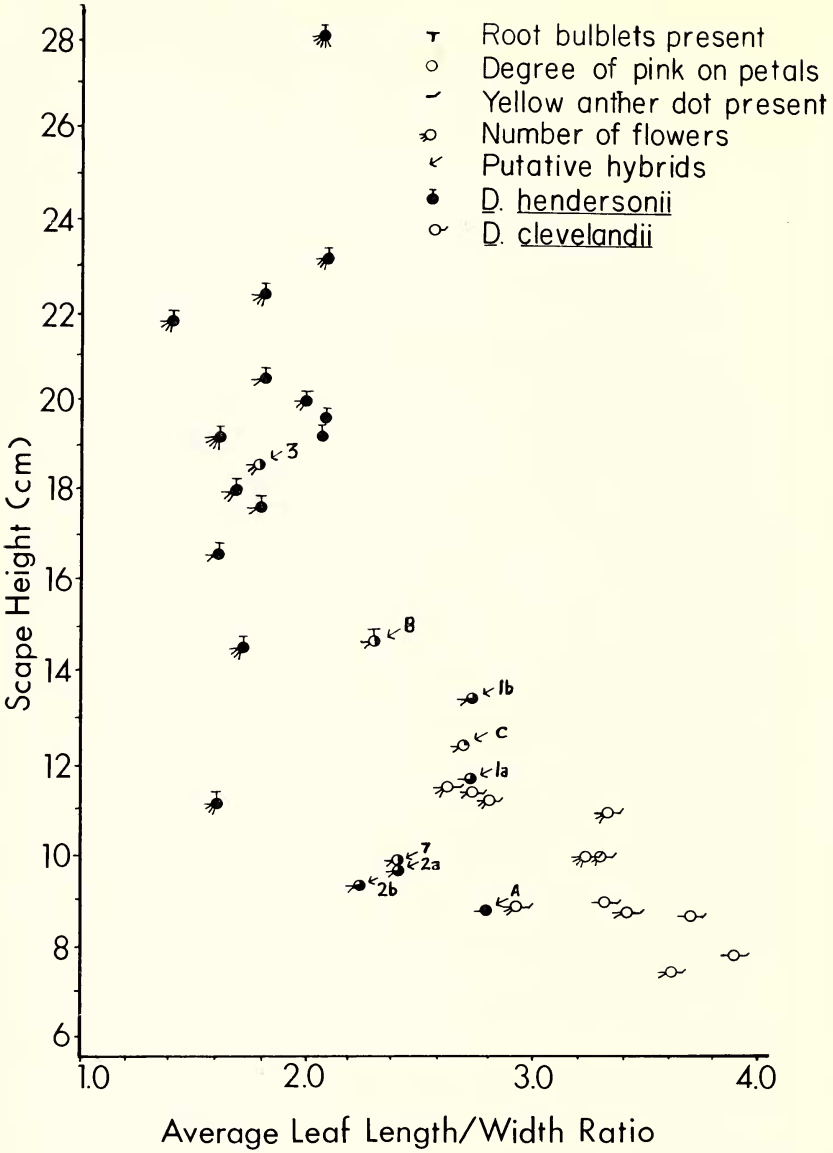


FIG. 1. Scatter diagram of population of *Dodecatheon* at the Table Mountain study site. Root bulblet data were not available for the putative hybrids other than plants A, B, and C, since measurements were made on living plants. Plants A, B, and C represent earlier collections, for which chromatographic data are not available.

tain site. This is in accordance with Thompson's (1953) work on this genus. It was not necessary for this study to establish that all plants of each species had $n = 22$; it was necessary only to establish that such

individuals were present at the Table Mountain site, since hybridization producing viable offspring is possible in this genus only between plants with the same chromosome number (Thompson, 1953).

Pollen stainabilities indicate that both species are highly fertile. *Dodecatheon hendersonii* was consistently high, with an average of 98.8 percent (SD ± 0.66), while *D. clevelandii* was somewhat lower and more variable (average 90.5 percent; SD ± 7.2). Percent stainabilities for the putative hybrids were 58 (plant 1a), 63 (1b), 8 (2a), 8 (2b), 82 (3), and 87 (7). Plants 4 and 6 were not sampled in this respect. These results correlate well with the observed frequency of collapsed or abortive grains.

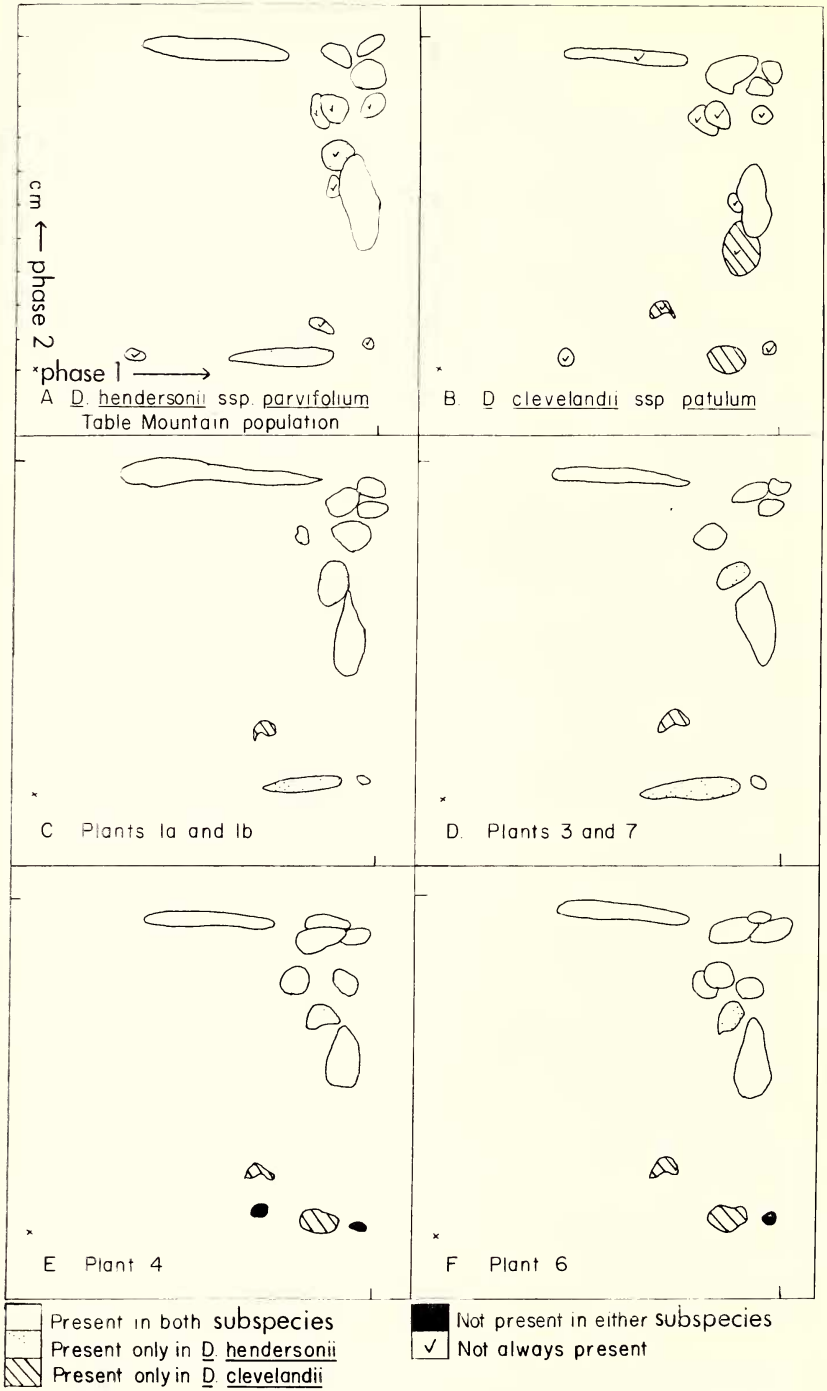
Drawings of chromatographs are shown in Figure 2. Some spots were consistently present for both species, some consistently present for just one species, and some were inconsistent, both in their presence and their species specificity. Some spots were pigmented, while others were visible only under ultraviolet light. For technical reasons, chromatographic data are not available for putative hybrids A, B, C, 2a, and 2b, nor morphological data for plants 4 and 6.

Discussion

In general, the putative hybrids tend to resemble *D. clevelandii* more than *D. hendersonii*, especially with regard to scape height and leaf shape (fig. 1).

Dodecatheon hendersonii and *D. clevelandii* have high pollen stainability and, hence, presumably high viability. They would not, therefore, be expected to have arisen recently as a result of hybridization. Putative hybrids, however, have significantly lower percentages, which supports the hypothesis of hybridization. Plants 1a and 1b, because of their similar morphology and adjacent growing sites, are thought to represent a clone, as do plants 2a and 2b. Pollen stainability results indicate that this is indeed the case. The similar results for each pair would indicate a genetic basis for the sterility, as would be expected in hybrid plants, rather than environmentally induced sterility (frost, nutrition, etc.).

Results of chromatography show that these two species have similar chromatographic profiles (fig. 2, A and B). There are a few species-specific compounds, but the majority of the compounds are shared by both, indicating that these species are closely related genetically. *Dodecatheon hendersonii* was found to be more variable between populations with regard to leaf compounds than was *D. clevelandii*, which was quite consistent chemically between populations. This may indicate a greater degree of genetic heterogeneity among populations of *D. hendersonii* in Butte County. Chromatographs of all the putative hybrids tested (fig. 2, C–F) showed the presence of at least one species specific compound from each species, in addition to compounds found in common with both species. The most probable way that these plants could have received such a combination of compounds is through hybridization between the two species. One of the species-specific compounds of *D. clevelandii* does not


cm ← phase 2
phase 1 ⟶
A D. hendersonii ssp. parvifolium
Table Mountain population
B D. clevelandii ssp. patulum
C Plants 1a and 1b
D. Plants 3 and 7
E Plant 4
F Plant 6
Present in both subspecies
Present only in D. hendersonii
Present only in D. clevelandii
Not present in either subspecies
✓ Not always present

show up in any of the hybrids (fig. 2); this may indicate that the genes coding for this substance need to be present in a homozygous condition in order for this chemical to be produced.

Some putative hybrids (fig. 2, E and F) showed the presence of compounds that were not found in either species. Alston and Turner (1963) showed the presence of *de novo* substances in hybrids of *Baptisia* (Leguminosae). These substances could be formed by small mutations or new gene combinations that were present in the putative hybrids but were not present in the two species. An alternative explanation is that they are substances that are present in one or the other species, but that they have been altered in some small way (possibly through hybridization) so as to show different chemical properties in the putative hybrids. Since no qualitative chemical analysis was done on any of these compounds, a definite conclusion concerning these particular spots cannot be drawn at this time.

The range of pollen stainabilities, variations in leaf chemistry, and degree of morphological overlap between the putative hybrids and the two species (fig. 1) may indicate that the putative hybrids do not all represent F_1 offspring. Since the majority of the putative hybrids were growing well within the *D. clevelandii* population, it is not unreasonable to suspect that introgression with the *D. clevelandii* parent has been and is occurring. This may explain why the putative hybrids tend to resemble *D. clevelandii* more than *D. hendersonii*.

Acknowledgments

I express my appreciation to Dr. Kingsley Stern for his constant encouragement and support during the preparation of this paper. This report is excerpted from a thesis submitted to the faculty of California State University, Chico, in partial fulfillment of the requirements for the Master of Arts degree in botany.

Literature Cited

Alston, R. E. and B. L. Turner. 1963. Biochemical systematics. Prentice-Hall, Englewood Cliffs, New Jersey.

Goodwin, J. F. 1973. Interspecific hybridization between two species of *Dodecatheon*. Master's thesis, California State University, Chico.

Hauser, E. J. P. and J. H. Morrison. 1964. The cytochemical reduction of nitro-blue tetrazolium as an index of pollen viability. Amer. J. Bot. 51:748–752.

Thompson, H. J. 1953. The biosystematics of *Dodecatheon*. Contr. Dudley Herb. 4:73–154.

Fig. 2. Chromatographs of two species of *Dodecatheon* and putative hybrids. Those for the two species are composite chromatographs (2A based on 5 plants; 2B based on 6 plants). In addition, the diagram for *D. hendersonii* is accurate for only the Table Mountain population (that for *D. clevelandii* is accurate for all populations sampled). Diagrams C through F represent single plants, unless otherwise noted. For composition of the solvent phases, refer to text.